Plant Tissue Cult. & Biotech. **16**(1): 31-36, 2006 (June)

PTC&B

In vitro Plant Production through Apical Meristem Culture of Bitter Gourd (*Momordica charantia* L.)

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Key words: Apical meristem, Bitter gourd, *In vitro,* Plant production

Abstract

The growth of meristem was observed on semisolid MS medium supplemented with 0.05 mg/l Kn + 0.1 mg/l GA_3. After three weeks, meristems were transferred to MS supplemented with BA, Kn, IBA, NAA and IAA singly or in combination for shoot elongation and root initiation. Among different treatments for shoot initiation with elongation were obtained in MS supplemented with 1.0 mg/l BA + 0.1 mg/l IBA + 0.3 mg/l GA_3. On the other hand good rooting was observed when 0.5 mg/l IBA and 0.1 mg/l NAA were used to fortify MS semisolid medium. Ten weeks old *in vitro* plantlets were successfully planted in soil through gradual acclimation.

Introduction

Momordica is a genus of herbaceous annual or perennial climbers with 45 species native to tropcial Asia and Africa. About six species are cultivated for their fruits used as vegetable, the most ocmmon being *Momordica charantia* L. The fruits are bitter but edible. Tender shoots, leaves and root tubers are also used as a vegetable and the seeds as condiment.

Cucumis virus - I and *III* is common on bitter gourd, bottle gourd, cucumber, watermelon and other cucurbits hosts (Brunt et al. 1996). The disease is charactrized by the formation of streaks in the inter-vernal regions of the leaves, which enlarge to form characteristic green vein-banding. Diseased plants flower scarcely or none and fruiting is very much reduced both in number and size (Rangaswami and Mahadevan 2002). Virus free plants can be obtained from five variable methods such as heat treatment followed by meristem culture. Adventitious shoots formation followed by meristem culture and grafting of meristems on virus free rootstocks or micro-grafting (Pierik 1997) are also practised. Among above methods, meristem culture technique may solve effectively. With this view in mind, the present investigation was undertaken to develop standard protocol for obtaining healthy disease free bitter gourd plants through meristem culture.

Materials and Methods

Two genotypes, namely large fruited BGGB1 and small fruited BGGB14 bitter gourd plants established in the Fruit Research Station, Binodpur, Rajshahi were used for culture initiation. Very young fresh shoot tips were carefully excised from the field-grown plants. Shoot tips were sterilized with a solution of 0.1 g/l $HgCl_2$ with constant stirring for 2 - 3 min followed by four rinses with autoclaved distilled water. Meristems with immature leaves and leaf primordia were snapped off. Then the excised meristems were cultured in semisolid MS supplemented with different kinds of growth regulators either singly or in combinations (Table 1). After three weeks, the established isolated meristems were subcultured on semisolid MS medium for five to six weeks using different concentrations of BA, GA_3, IBA and NAA either singly or in combinations for shoot elongation and root formation. All cultures were incubated at 25 ± 2°C under 16 h photoperiod. The medium was semisolidified with 6 g/l of agar and sucrose was used. The pH of media was adjusted at 5.8 before autoclaving. After ten weeks, healthy plantlets with leaves and roots were successfully planted in soil through gradual acclimation.

Results and Discussion

Isolated meristems of two bitter gourd genotypes were cultured on MS supplemented with different concentrations of growth regulators and their responses are presented in Table 1. Successful responses of meristems depend on the removal of exogenous and endogenous contamination. So, an experiment was designed for standardizing exact duration of time for surface sterilization. For this purpose 0.1% $HgCl_2$ solution was used at different time interval *viz.* 2, 3, 4, 5, 6, 7 and 8 min. Among the treatments, 95% cultures were found to be contamination free, when they were treated for 3.5 min and 75% cultures were found to be contamination free (data not shown). Using the same concentration of $HgCl_2$ contamination free culture were observed in other plants (Mallick and Roy 2001; Roy et al. 1994; Maity et al. 2001).

The cultured isolated meristems, commenced their initial growth by increasing in size and gradually changed to light green in colour within six - ten days. Pattern of morphogenic dedifferentiation of cultured isolated meristems of two varieties was more or less similar.

The results presented in Table 1 show that maximum response was observed for both varieties when the meristems were cultured on MS with 0.05 mg/l Kn + 0.1 mg/l GA_3. Good response was also observed when MS contained 1.0 mg/l GA_3 as growth regulator (Fig. 1). Ahmed et al. (2000) reportd that liquid MS0 and MS with 0.1 mg/l GA_3 were best for early establishment of tomato meristems. The high percentage of primary establishment of meristems of brinjal was

observed on MS + 2.0 mg/l BAP by Nasir (2004). GA_3 alone or in combination with cytokinin has been extensively used for primary establishment of meristem culture in different plant species such as *S. tubersoum*, *D. hybrida* (Morel and Martin 1952). BAP was found the most effective cytokinin commonly used in meristem culture (De 1992). These results are in agreement with those previously reported by Mederos-Molina (2002) that the combination of 1.44 µM GA_3, 2.2 µM BAP and 1.1 µM IAA was the best for developing shoots in *Maytenus* sp. Ahmed et al. (2000) reported that the treatment MS + 0.5 mg/l BA and MS + 0.5 mg/l Kn showed good responses for shoot initiation and elongation in tomato. Nasir (2004) reported that MS + 0.5 mg/l GA_3 was the best combination on shoot elongation in brinjal.

Table 1. Effect of growth regulators in MS medium on primary responses of isolated meristems of bitter gourd.

Conc. of GR (mg/l)	Shoot response	
	Large fruited (%)	Small fruited (%)
Kn + GA_3		
0.01 + 0.1	15	10
0.02 + 0.1	20	14
0.03 + 0.1	20	16
0.04 + 0.1	30	20
0.05 + 0.1	70	63
0.0 + 0.1	15	10
0.0 + 0.5	50	45
0.0 + 1.0	65	61
BAP + NAA		
0.1 + 0.0	20	10
0.5 + 0.0	30	25
1.0 + 0.0	50	50
1.0 + 0.1	55	60
1.3 + 0.1	55	60
BAP + IBA		
1.0 + 0.1	60	45

After three weeks the established primary meristems were aseptically taken out and subcultured on semisolid MS supplemented with various concentrations of growth regulators either singly or in combination for shoot initiation and elongation. Average length of the longest shoot, percentage of explants showing elongation were recorded and are shown in Table 2. Among different combinations the media with 1.0 mg/l BAP + 0.1 mg/l IBA + 0.3 mg/l GA_3 was the best formulation for shoot elongation of both the varieties (Fig. 2).

After seven weeks these shoots were transferred to a rooting medium supplemented with various types of auxin. In case of root formation the medium with 0.1 mg/l NAA and 0.5 mg/l IBA was found to be the best formulation for bitter goured (Fig. 3). Efficiency of IBA in root induction was also observed in *Vigna mungo* (Hoque et al. 1984) and grape (Chakravorty 1986).

Table 2. Effect of cytokinin and auxin in semisolid MS on shoot elongation from primary established meristems of bitter gourd.

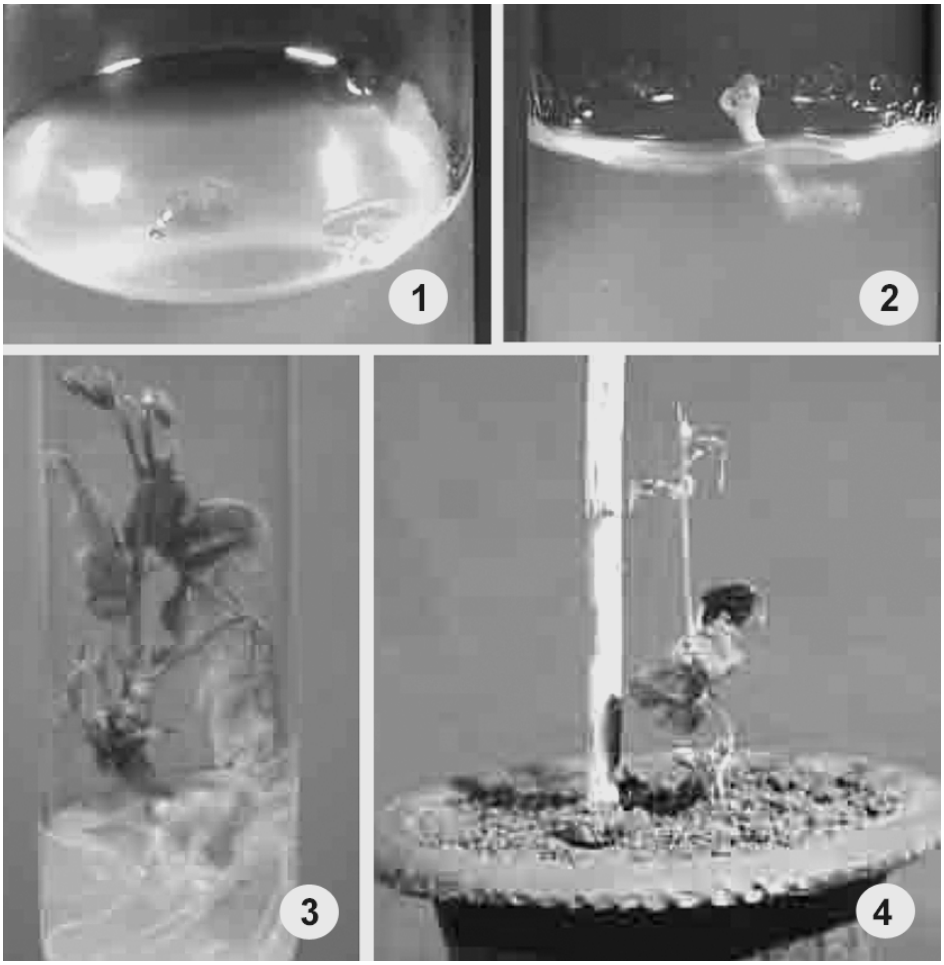
Growth regulators (mg/l)	Shoot length (cm)	
	Large fruited	Small fruited
BAP + NAA + IBA + GA_3		
1.0 + 0.0 + 0.0 + 0.0 + 0.0	2.5 ± 0.25	2.5 ± 0.20
1.5 + 0.0 + 0.0 + 0.0 + 0.0	2.9 ± 0.05	2.7 ± 0.23
1.0 + 0.1 + 0.0 + 0.0 + 0.0	3.0 ± 0.5	3.0 ± 0.4
1.5 + 0.1 + 0.0 + 0.0 + 0.0	3.0 ± 0.4	3.0 ± 0.4
1.0 + 0.0 + 0.1 + 0.0	3.0 ± 0.6	3.0 ± 0.6
1.0 + 0.0 + 0.1 + 0.0	3.0 ± 0.8	3.0 ± 0.7
1.0 + 0.0 + 0.1 + 0.1	3.6 ± 0.6	3.7 ± 0.6
1.5 + 0.0 + 0.1 + 0.1	3.5 ± 0.9	3.7 ± 0.7
1.0 + 0.0 + 0.1 + 0.2	4.9 + 0.25	4.9 + 0.5
1.0 + 0.0 + 0.1 + 0.3	5.0 ± 0.25	5.0 ± 0.1

Table 3. Effect of auxin in semisolid MS on root formation in *in vitro* established shoot form meristem of bitter gourd.

Growth regulators			
Auxin	Amount (mg/l)	% of response	No. of roots/shoot
IBA	0.1	40	3.2
IBA	0.5	80	7.6
IAA	0.1	15	5.2
IAA	0.5	50	6.9
NAA	0.1	95	10.9
NAA	0.5	25	8.1

After ten weeks, the well rooted plantlets were taken out very carefully from test tubes and roots were washed gently and transplanted to small polybags containing sterilized soil. They were covered with transparent polythene bags to maintain high humidity and kept in the growth chamber for seven days. Within seven days the plantlets began to form new leaves and resumed fresh growth. Plantlets were successfully acclimated with natural condition through gradual

increase of duration of exposure to sunlight. After acclimatization, the plantlets were transplanted to soil (Fig. 4).



Figs. 1-4: 1. Differentiated meristem with leaf primordia in MS semisolid medium supplemented with 0.1 mg/l GA_3 + 0.05 mg/l Kn. 2. Shoot induction from *in vitro* established meristem on MS semisolid medium with 1.0 mg/l BAP + 0.1 mg/l NAA. 3. Root induction from *in vitro* established meristem derived shoot on MS semisolid medium with 0.1 mg/l NAA. 4. Meristem derived plant in pot.

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